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NONRESPIRABILITY OF CARBON FIBERS IN RATS FROM REPEATED INHALATION EXPOSURE

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13. ABSTRACT *(Maximum 200 words)*
Carbon fibers are light weight, high tensile strength synthetic fibers widely used in aircraft, and as the applications for carbon fiber expand, so does the probability of worker exposure via inhalation and skin contact. Although there have been numerous in vitro and in vivo studies addressing the health hazards from carbon fiber exposure, few inhalation studies have been conducted. The purpose of this study was to determine if the 3.5 μm diameter carbon fibers were respirable and if there were any deleterious upper respiratory or irritant effects from repeated exposure. Groups of male Fischer 344 rats were exposed by inhalation to three concentrations of carbon fibers for 1 hr per day for 9 days with one weekend without exposure. Sonic nozzle air-exposed rats served as the control. Exposed rats and respective groups of controls were submitted for lavage and biochemical, physiological and pathological evaluation at 24 hr, 14 days, and 3 months post-exposure (PE). Scanning electron microscopy (SEM) was conducted on lungs from rats submitted at 0, 24 hr, and 14 days PE. Results from all the biological endpoints were negative and microscopic evaluation of the nasal turbinates, trachea, and lungs indicated the carbon fibers were not respirable.

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PREFACE

The work described in this report was authorized under Project No. 1L162622A552, Smoke and Obscurants. The work was started in July 1989 and completed in November 1989. The experimental data are contained in laboratory notebooks 85-0162, 88-0117, and 89-0062.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals" as promulgated by the committee on Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council. This study was consistent with Good Laboratory Practice and was conducted in accordance with protocol #22089000A243 approved by the CRDEC Laboratory Animal Use Review Committee.

The use of trade names or manufacturers' names in this report does not constitute an official endorsement of any commercial products. This report may not be cited for purposes of advertisement.

Acknowledgments

The authors thank David Burnett, Bernardita P. Infestio, and Elizabeth Lawrence-Beckett, Toxicology Division, CRDEC, for their help in the analyses of the lavage fluid. They thank Erica R. Riley, Physics Division, CRDEC, for her SEM analysis of the generated material and Dr. Lucas Brennecke, Pathology Associates, for his histopathological evaluations. Thanks are also extended to Dottie Berg, Toxicology Division, CRDEC, for her assistance with manuscript preparation.

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1. INTRODUCTION

There are numerous commercial applications for carbon fibers such as sports equipment, reinforcing materials in structural composites, and prosthetic devices for humans.[1,2] Carbon fiber can be synthesized from polyacrylonitrile (PAN) or from petroleum pitch.[3] PAN-based fibers are the purer, more commonly used precursor and will be the material used in this study. Utilization of carbon fibers in military aircraft has increased because of the advantage of lightweight strength and smooth outer construction.

Although there are few inhalation studies addressing the health hazards from carbon fiber exposure, several *in vitro* and *in vivo* studies have been conducted comparing PAN and pitch based carbon fibers.[4] Mutagenicity tests with extracts of pitch-based carbon fibers elicited positive results in sister chromatid exchange (SCE) and unscheduled DNA synthesis (UDS) tests, and negative results in the chinese hamster ovary (CHO) and Ames tests. Extracts of PAN-based carbon fibers were negative in all these tests. Likewise, the pitch-based carbon fiber extracts produced positive results in a lifetime painting study in mice. Negative results were obtained with extracts from PAN-based carbon fibers in similar carcinogenicity testing in mice.[5] Implant studies in rabbits, rodents and humans have resulted in little or no significant tissue reactions.[6]

In the hamster tracheal organ culture model, graphite fibers were compared to crocidolite asbestos and no significant cellular differentiation changes occurred after 1 and 3 weeks in culture; whereas, asbestos produced significant proliferative degenerative changes.[7] Limited epidemiologic studies of carbon fiber production workers have shown no adverse pulmonary effects except for some minor skin irritation.[8]

Modeling experiments based on aerodynamic equivalent diameters have demonstrated that diameter is the determinant of respirability.[9] The limits of respirability for fibers is 3.5 µm diameter.[10,11] The industry standard for carbon fibers is 7-8 µm diameter which is outside the respirable range. Intratracheal and inhalation studies on carbon fibers and dusts have not resulted in any deleterious changes; however, in one inhalation study the aerosol generated was particulate dust and not fibers.[12,13] In another subchronic inhalation study, rats were exposed to only one concentration of fibers, thus preventing a dose-response evaluation.[14] In this study it will be determined if the 3.5 µm diameter carbon fibers are respirable and if there are any deleterious upper respiratory or irritant effects from repeated exposure.

2. MATERIALS AND METHODS

2.1 Materials.

The PAN-based, 3.5 µm diameter carbon fibers used were obtained from Hercules Inc.. These were unsized fibers composed of 92-99.7% carbon according to the material safety data sheet provided. The aspect ratio of the fibers was approximately 1000.

2.2 Experimental Design.

Groups of male Fischer 344 rats were exposed by inhalation to three concentrations of carbon fibers for 1 hr per day for 9 days with one weekend without exposure. Sonic nozzle air-exposed rats served as the control. Fischer 344 rat was the species of choice because it had been used in previous studies with particulates and there is an extensive data base for comparison. Exposed rats and respective groups of controls were submitted for lavage and biochemical, physiological, and pathological evaluation at 24 hr and14 days post-exposure (PE). Scanning electron microscopy (SEM) was conducted on lungs from rats submitted at 0 hr, 24 hr, and 14 days PE according to the following schedule:

NUMBER OF RATS

	Pathology		Lavage & Physiology		SEM	
	24 hrs	14 da	24 hrs	14 da	0,24	14 da
CONTROLS	6	6	6	6	0, 3	3
EXPOSED						
Carbon Fibers						
100 mg/m^3	6	6	6	6	3, 3	3
60 mg/m^3	6	6	6	6	0, 0	0
20 mg/m^3	6	6	6	6	0, 0	0

Separate animals were required for pathology and lavage, because necropsy procedures are terminal; whereas, physiology was conducted on the same animals used for lavage. Separate animals were also required for SEM as the trachea and entire lung must be perfused with a different fixative. Previous experiments have shown that six rats per group are the minimum number for statistical significance for lavage and physiology, while three are sufficient for SEM. Only high dosage and control rats were evaluated with SEM because it was expected that the lower concentrations would produce a no effect level.

2.3 Animal Husbandry/Necropsy.

The rats were housed in stainless steel suspended cages in racks in the animal care facility of the Toxicology Division. They were housed under uniform light (12 hr light/12 hr dark sequence), temperature (22 ± 2 °C) and humidity (30-70%) controlled conditions. Certified commercial rodent chow and water were available *ad libitum*, and cage trays were changed thrice weekly. The rats were transported to and from the inhalation chamber in a climate controlled van.

Animals were randomized, weighed, and tatooed one week prior to exposure. An on-call veterinarian was available if the animals were to suffer undue distress or disease processes during the course of the experiment. Maintenance and use of animals was in accordance with the guidelines contained in Guide for the Care and Use of Laboratory Animals.[15] The animals were observed for any abnormal activity daily and were weighed at weekly intervals during the experimental and PE periods.

At the end of the experimental period, all scheduled rats were euthanized with carbon dioxide, necropsied, and their tissue prepared for light microscopic examination by Pathology Associates Inc., Frederick, MD , in accordance with Contract No. DAAA15-88-D-0012. During necropsies, the animal total body weight was recorded. The remaining scheduled rats were physiologically evaluated and lavaged by the Toxicology Division, Chemical Research, Development and Engineering Center, as detailed below. Pathology Associates inc. evaluated the tissues for histopathologic changes.

2.4 Physiological Evaluations.

Lung lavage and pulmonary physiological testing were performed on the same animal to enable correlation of biochemical changes with functional changes. At 24 hr, 14 days, and 3 months PE, the rats were anesthetized intraperitoneally with sodium pentobarbital (40 mg/kg), and a tracheal catheter was connected to a Fleish® pneumotachometer for the measurement of respiratory flow. An air-filled esophageal catheter was inserted into the esophagous approximately to the level of the thoracic inlet and was connected to one arm of a Hewlett-Packard® differential pressure transducer for the measurement of esophageal pressure. Transpulmonary pressure (the difference between esophageal pressure and airway pressure derived from a lateral tap at the distal end of the endotracheal tube) is used for all calculations. Both flow and pressure signals were processed in a Buxco Electronics®, Inc. Pulmonary Function Computer and the following parameters were recorded on a Buxco Data Logger: flow, tidal volume, transpulmonary pressure, compliance, and resistance. Compliance, measured by the ratio of the volume change in a tidal breath to the pressure change between end expiration and end inspiration, is a standard physiological method of assessing the overall elasticity or distensibility of the lungs and thorax. Restrictive pulmonary diseases (e.g. fibrosis, silicosis) result in decreases in compliance due to a stiffening effect which increases the work of breathing. Resistance is a measure of the pressure difference required for a unit flow change. Inhalation of dusts/fibers may lead to an increase in airway resistance. Both compliance and resistance were measured as indicators of functional impairment.

2.5 Bronchoalveolar Lavage (BAL).

Immediately following the pulmonary measurements, the esophageal catheter was removed and the lavage procedure commenced. The lung washing technique consisted of instilling a calculated volume of normal saline (0.015 mL/g body weight) into the lung and immediately withdrawing the saline until a slight pressure is felt on the syringe plunger. Two lavage washes were done in quick succession. The recovered lavage fluid from both washes was pooled and centrifuged at 300 g for 10 min at 4 °C.

Following centrifugation, the fluid was separated into supernatant and pellet fractions. The pellet was resuspended in 1 mL 50% bovine serum albumin and total cell counts were taken on a ZBI Coulter Counter®. A differential cell count was made using a modified Pap staining method. The cell pellet was resuspended in Hank's buffered saline; the macrophage concentration was determined in a hemocytometer and cell viability determined via the trypan blue exclusion test.[16] The supernatant lavage fluid was assayed for total protein with the Bio Rad® Protein Assay and for enzymatic activity of lactate dehydrogenase (LDH), alkaline phosphatase (ALKP), and ß-Glucuronidase (ß-Glu) LDH and ALKP were determined on an Abbott VP Series II using an Abbott Analysis Kit and ß-Glu was assayed using a Sigma Chemical Co. kit.

2.6 Special Studies (Scanning Electron Microscopy).

Scanning electron microscopy (SEM) has been used to observe the deposition pattern of a number of inorganic particles in the lungs of rats and mice. The particles studied were aerosolized dusts of chrysotile and crocidolite asbestos, fiber glass, alpha-quartz, and ash from Mt. St. Helens. The particles were found at the bifurcations of alveolar ducts preferentially over individual alveolar spaces and along alveolar duct surfaces.[17] SEM analysis would also be applicable to this study and Dr. David Mattie of the Ultrastructural Research Laboratory, Armstrong Aerrospace Medical Research Laboratory, Wright-Patterson AFB, OH, conducted the SEM evaluation according to his published procedures. These procedures were used to examine lungs after instillation of reduced diameter carbon fibers[18] and to observe changes in lungs after administration of 4-ipomeanol.[19]

2.7 Chamber Operation and Sample Collection.

The rats were exposed in a 3.3 m^3, plexiglass, aerosol test chamber located in Building E5951. The carbon fibers were delivered to the intake of the chamber via a sonic nozzle which was operated at a pressure of 30 psi. The concentration in the chamber was maintained by varying the chamber flow and nozzle pressure. Continuous monitoring of the aerosol concentration was conducted with a class III, medium power Helium/Neon laser and with radar transmissometers. The output of each device was measured by a strip chart recorder and by a computer controlled data acquisition system. From these measurements, the concentration was calculated using the geometric optic inversion technique.[20] Prior to the start of exposures, calibration of the chamber was conducted to assure a stable concentration. Previous work with this material had shown what the laser transmission readings had to be to correspond to the test concentrations; a chart of this information is shown in Figure 1. This calibration chart provided the nozzle operator with the information to disseminate more or less material to achieve the selected concentrations. Filter samples of the disseminated aerosol were also analyzed by electron microscopy for particle size characterization. Pre-filtered, conditioned, room air was the air source, and the temperature and humidity of the chamber were maintained at 22 ± 2 °C and 30-70%, respectively (in accordance with Organization for Economic Cooperation and Development guidelines). The airflow was maintained to insure a chamber oxygen content of at least 19%. The exhausted air was filtered through another particulate filter.

2.8 Data Analysis Plan.

Data analysis was conducted according to a statistical "decision tree".[21] First, Bartletts's Test for homogenicity of variance was used as a check of the assumption of equivalent variances, followed by the use of analysis of variances (ANOVA). Non-parametric, heterogeneous data was analyzed by the Kruskal-Wallis non-parametric ANOVA. Finally, Dunnett's Test was used on parametric homogeneous data to identify significantly different groups.

This study was consistent with Good Laboratory Practice (GLP). Every reasonable attempt was made to control bias throughout the experiment. Because there was dust deposited on the high-dosed animals, it was obvious to the technicians recording toxic observations which animals were exposed. However, during physiological evaluation and lavage, the animals were taken on rotation; one from each group rather than one whole test group at a time. All chamber analysis data, toxic observations, and animal weights were recorded in official CRDEC notebooks. Lavage and physiological data were generated on hard copy outputs from automated equipment. This data was entered into and analyzed in official CRDEC notebooks. All other associated raw data (statistical printouts, necropsy incidence tables, etc.) were stored in the Toxicology Division, Research Directorate, CRDEC, archives.

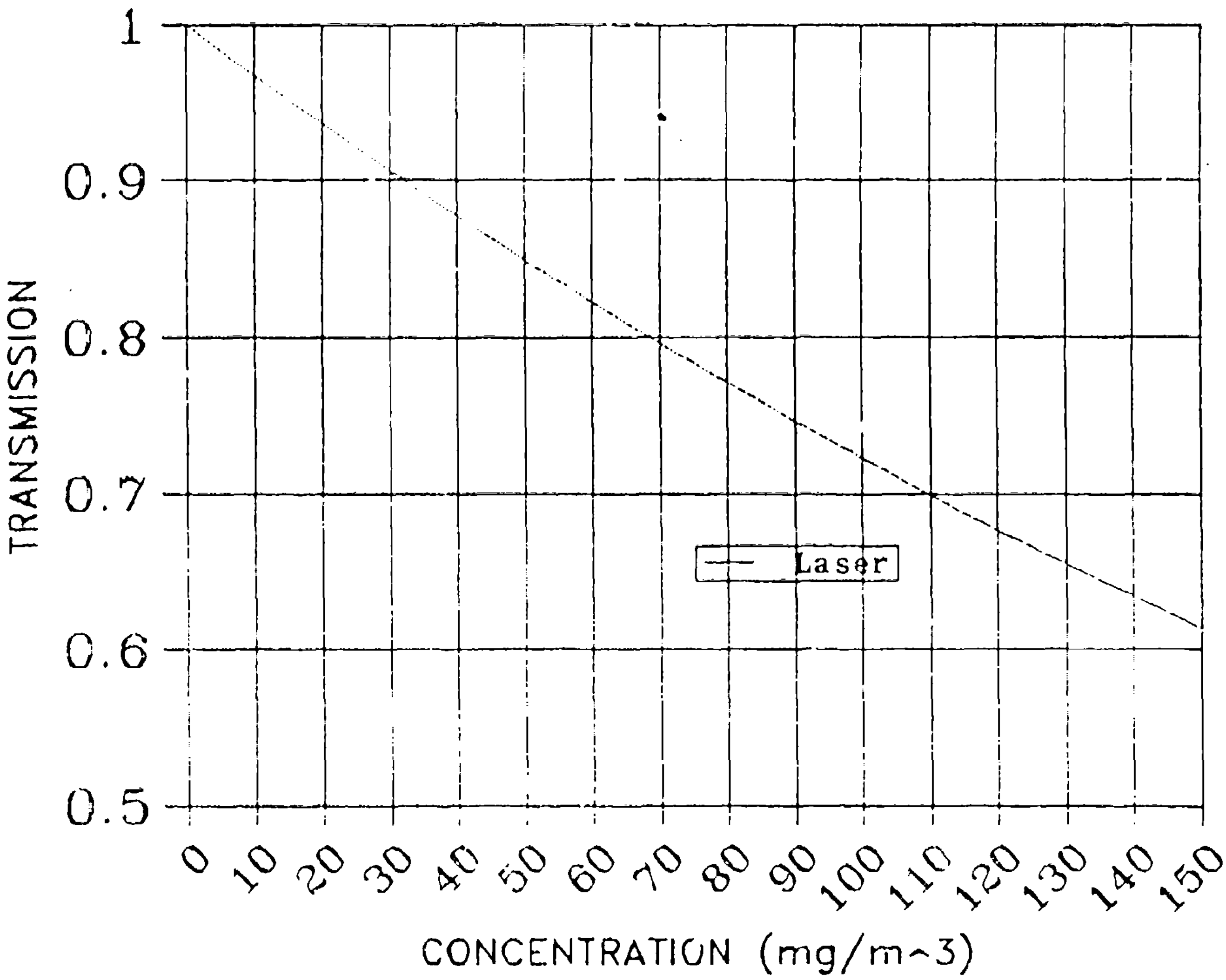

Figure 1. Concentration vs. Transmission

3. RESULTS

3.1 Chamber Analyses.

Preliminary tests with the material before the actual exposures showed that, when the aerosol was present in the chamber for an hour, particles of the material coated the windows of the chamber through which the laser beam was passing. This had the effect of moving the baseline or, as it is sometimes referred to, the 100% line down to 90% after an hour. This shift did not appear to be related to aerosol concentration, but only to time. This shift was taken into consideration during the actual exposures, by lowering the predetermined transmission level 1% every 6 min. The average concentration for each of the exposure levels was 22.2 $\pm$ 3.9 mg/m^3, 58.3 $\pm$ 3.6 mg/m^3, and 103.5 $\pm$ 5.1 mg/m^3. The SEM particle size analysis of collected samples showed that the diameters and lengths were not altered by the sonic nozzle dissemination technique (Figure 2). The dust which appears in the photo along with the fibers was examined using an energy dispersive system, which is part of the electron microscope. These particles were primarily dust and were found to be present even on samples taken with neither aerosol material nor animals in the chamber. Presumably, airborne dust impacted and stuck to the tape preparation and handling.

3.2 Physiological and BAL Effects.

None of the animals exhibited any adverse effects at any of the exposure levels. Following exposure, the rats conducted normal preening, eating, and drinking; there was normal weight gain in all groups. There were no statistically significant differences in pulmonary physiology and BAL parameters (Tables 1-3).

3.3 Pathological and SEM Evaluations.

Gross and microscopic examination of all tissues of the control and exposed rats revealed no treatment-related lesions in either the 24 hr or 14 day PE groups. No carbon fibers were present in any tissues. This lack of carbon fibers was confirmed by SEM analysis of the turbinates, trachea, and lungs of the high dosage (100 mg/m^3) exposed rats. Because the carbon fibers were not respirable, it was decided that there was no need to examine the 3 month PE groups and the protocol was ammended to delete those groups (Appendix B).

4. DISCUSSION

It was not surprising that the 3.5 µm diameter fibers were not respirable since the aerodynamic equivalent diameter (Dae) is the determinant of respirability where Dae is defined as the diameter of a unit density sphere having the same terminal settling velocity as a given particle. If Dae is less than 10 µm then it can be respirable; however, the majority of particles deposited in the alveoli are from 0.8 to 3.0 µm.[22] A fiber which has an actual diameter of 3.5 µm has very little probability of reaching the alveoli regardless of how short it may be. As the length increases, the Dae increases and alveolar deposition decreases.[9] For example, if a fiber had an actual diameter of 3.5 µm, its Dae would be 7.8 µm if its length were 10 µm. If the fiber length increased to 70 µm, that same fiber would then have a Dae of 10.4 µm; thus, a 3.5 µm fiber has very little probability of alveolar deposition. This theory is supported by studies on the size analysis of particles found in human lungs exposed to asbestos fibers. These studies indicate that the upper limits of respirable fibers are either 3.5 µm in diameter or 200 µm in length.[23] Similar upper

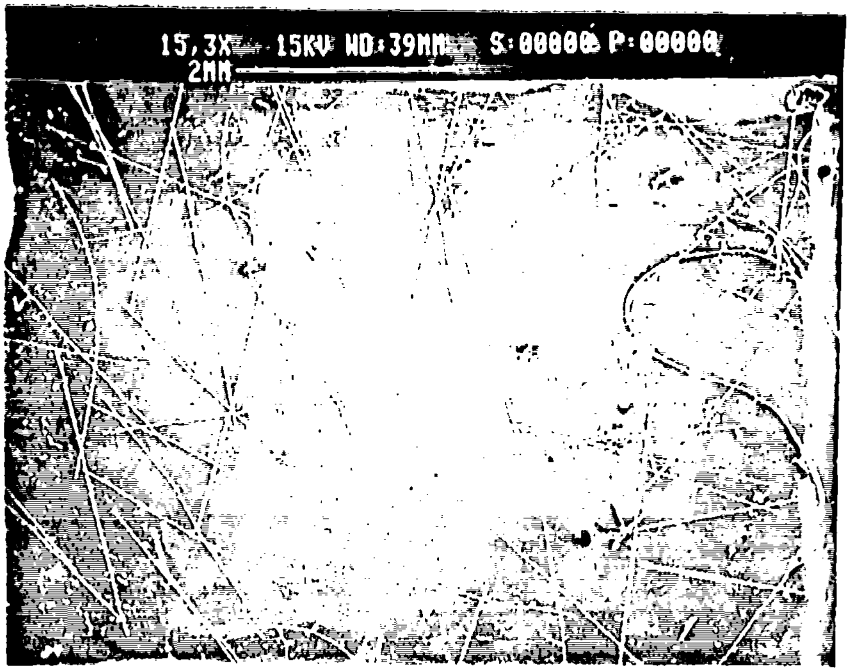

Figure 2. SEM Photo of Generated Fibers.

TABLE 1. Biochemical Results From Analysis of Lavage Fluid From Rats Exposed to Carbon Fibers.*

24 Hr Post Exposure

Exposure Group	β-gluc (Sigma μ/mL)	LDH (IU/L)	Protein (μg/mL)	Alk Phos (IU/L)
Control	4.3 ± 2.2	61 ± 13	467 ± 61	68 ± 18
20 mg/m^3	2.5 ± 0.9	52 ± 17	427 ± 92	54 ± 16
60 mg/m^3	2.7 ± 0.6	46 ± 11	540 ± 229	56 ± 10
100 mg/m^3	2.8 ± 0.6	50 ± 9.2	471 ± 127	62 ± 13

14 Day Post Exposure

Exposure Group	β-gluc (Sigma μ/mL)	LDH (IU/L)	Protein (μg/mL)	Alk Phos (IU/L)
Control	7.7 ± 2.1	44 ± 18	748 ± 94	70 ± 13
20 mg/m^3	6.3 ± 1.3	26 ± 3	1028 ± 283	61 ± 9
60 mg/m^3	7.5 ± 1.8	32 ± 1	812 ± 92	57 ± 12
100 mg/m^3	7.2 ± 1.4	32 ± 2	955 ± 289	50 ± 14

* Each value represents mean ± SD (n = 6), tested using Bartlett's Test and ANOVA @ $P \leq 0.05$.

Table 2. Cytological Results from Analysis of Lavage Fluid from Rats Exposed to Carbon Fibers.

24 Hr Post Exposure

Dose		Cell Count WBC $\times 10^3$	Total $\times 10^4$	Differential Count Macrophages %	Lymphocytes %	Neutrophils %
Control						
0.0mg/m^3	mean	3.90	5.37	99	1	- - -
	SD	0.85	1.46	1	1	- - -
Low						
20 mg/m^3	mean	3.77	4.52	97	3	- - -
	SD	1.23	1.98	2	1	- - -
Mid						
60 mg/m^3	mean	2.25	4.03	96	2	2
	SD	0.31	0.32	2	1	1
High						
100 mg/m^3	mean	2.33	4.04	98	2	- - -
	SD	0.84	0.93	1	1	- - -

14 Day Post Exposure

Dose		Cell Count WBC $\times 10^3$	Total $\times 10^4$	Differential Count Macrophages %	Lymphocytes %	Neutrophils %
Control						
0.0 mg/m^3	mean	2.52	1.89	98	1	1
	SD	1.24	0.63	1	1	- - -
Low						
20 mg/m^3	mean	2.27	3.70	97	3	- - -
	SD	0.59	1.50	2	1	- - -
Mid						
60 mg/m^3	mean	1.68	2.60	96	3	1
	SD	0.40	0.73	2	1	- - -
High						
100 mg/m^3	mean	1.70	2.53	96	3	- - -
	SD	0.43	1.64	2	2	- - -

Table 3. Physiological Results from Rats Exposed to Carbon Fibers.

24 Hr Post Exposure

Group	Weight (g)	Flow	Pleural Press.	Tidal Vol.	Compli- ance	Resis- tance	Resp. Rate	Minute Volume
Control	237	18.5	6.15	1.99	0.404	0.169	99.2	216
SD ±	14	11.9	1.17	1.23	0.317	0.071	28.9	142
20 mg/m^3	223	19.0	7.86	2.05	0.305	0.157	76.7	161
SD ±	5	7.0	1.23	0.97	0.143	0.051	17.2	54
60 mg/m^3	233	24.8	6.79	2.59	0.411	0.143	89.4	250
SD ±	5	12.3	1.18	1.30	0.188	0.067	11.6	127
100 mg/m^3	232	22.9	5.83	2.27	0.382	0.104	95.4	226
SD ±	6	15.9	0.97	1.59	0.162	0.037	29.2	144
Bartlett's Test	NS	NS	NS	NS	NS	NS	NS	NS
ANOVA	NS	NS	SIG	NS	NS	NS	NS	NS
Dunnett's Test	---	-	Ctvs20 = SIG Ctvs60 = NS Ctvs100 = NS	---	---	---	---	---

14 Days Post Exposure

Group	Weight (g)	Flow	Pleural Press.	Tidal Vol.	Compli- ance	Resis- tance	Resp. Rate	Minute Volume
Control	261	16.4	5.49	1.92	0.439	0.114	101.3	211
SD ±	9	4.7	0.82	0.47	0.178	0.063	14.4	25
20.0 mg/m^3	262	19.2	6.59	2.18	0.429	0.118	92.9	225
SD ±	17	3.6	2.37	0.81	0.263	0.067	27.0	124
60.0 mg/m^3	261	17.9	6 01	2.06	0.425	0.128	101.7	232
SD ±	11	7.4	1.68	0.71	0.202	0.056	11.1	92
100 mg/m^3	269	18.0	6.29	2.03	0.384	0.114	109.2	247
SD ±	11	5.9	1.64	0.73	0.187	0.058	24.6	109
Bartlett's Test	NS	NS	NS	NS	NS	NS	NS	SIG
ANOVA	NS	NS	NS	NS	NS	NS	NS	NA
Kruskal-- Wallis Non- Parametric ANOVA	---	---	---	---	---	---	---	NS

limits are seen with rodents exposed to asbestos, fiberglass, or mineral fibers. The retention of fibers less than 0.5 µm in diameter is significantly higher than fibers of larger diameters, with a peak of 7.6% and a fiber length of 21 µm. Fibers 1.0 µm in diameter with a fiber length of 5 µm had a maximum retention of 1%.[24] In deposition studies of glass fibers in rats, Morgan et al.[25] concluded that fibers with diameters exceeding 2 µm and with aspect ratios greater than 10 would be virtually nonrespirable to rats. It is generally assumed that the corresponding value for man is 3 µm diameter.

Few inhalation studies specifically conducted on carbon fibers can be found in the literature. Holt and Horne[12,13] conducted several inhalation studies on dust from carbon fiber. Guinea pigs were exposed for 7 to 104 hr to PAN-based chopped fibers described as RAE type 2. The chopped fibers had been further reduced by a hammer mill resulting in an aerosol of 98.8% nonfibrous particles (1 µm diameter) and 1.2% fibers of varying dimensions: 10 µm diameter with lengths greater than 100 µm; fibers 1 to 2.5 µm diameter with lengths up to 15 µm; and transparent fibers 1.5 µm diameter with lengths up to 30 µm. Their results showed that macrophages readily phagocytized the dust particles even up to 100 days following exposure. The few fibers seen were still extracellular after 27 weeks and no pathological effects were seen from carbon fiber dust in any of their experiments. This slow, continual dust clearance is similar to the studies on graphite dust reported by our laboratory.[25,26] We also found a continual macrophage clearance of graphite dust 3 months following acute and repeated exposures with no apparant adverse pathology.

Only one inhalation study has been published[14] in which carbon fibers were actually generated and comprised the test aerosol. This was a single concentration (20 mg/m^3) subchronic study. Rats were exposed to pulverized Celion, PAN-based carbon fiber for 6 hr/day, 5 days/week for 16 weeks. Rats were killed at 4, 8, 12, and 16 weeks of exposure and after a 32-week PE recovery. Exposed rats were compared to air-exposed controls for pulmonary function and histopathological change. There were no consistent, significant pulmonary function changes and no evidence of fibrosis or inflammation. Alveolar macrophages were seen containing fiber particles. The aspect ratio of the generated particles was reported to be 20 - 60 µm long with 7 µm diameters. Fiber length was determined by gently tapping one filter onto a glass slide and counting the number of fibers in various size ranges. Although this study did not show any deleterious effects from inhalation of carbon fibers, there are several unanswered questions in the experimental design. The sampling techniques did not measure the aerodynamic equivalent diameter; in fact, the method described probably missed any smaller diameter or shorter length fibers that may have been present in the pulverized aerosol. The gravimetric concentration did not represent a respirable concentration which was probably lower than the measured 20 mg/m^3. All of the published modeling experiments by Timbrell,[11] Harris,[27] and studies by Morgan,[24] and Hammad[23] question the respirability of a 7 µm diameter fiber. Although this was a single dose study and no dose response effects could be evaluated, it did demonstrate that inhalation of low concentrations of PAN-based carbon fibers did not result in any adverse pathological changes in rats.

Another single dose industry sponsored inhalation study on 3 µm diameter carbon fiber has been conducted and recently reported.[28] In this study, rats were exposed to 0 and 20 mg/m^3 chopped PAN-based carbon fibers with no surface sizing for 6 hr/day, 5 days/week for 16 weeks. There was no evidence of longitudinal splitting despite the chopping and pin milling.
Ten rats were sacrificed every 4 weeks during the exposure period and at 48 weeks and 80 weeks PE. There were no consistent treatment-related changes in any of the parameters evaluated: mortality, clinical condition, body weight, organ weight, organ to body weight ratios, pulmonary function, or histopathology. Particles of test material were seen in the pulmonary macrophages and the mucociliary and lymphoid clearance systems at all times It was not possible to determine from

the sections the depth of pulmonary penetration of true fibers; the macrophages contained only carbon dust. The method of dissemination most likely resulted in some horizontal cutting (length reduction) and/or there was possibly some *in vivo* reduction of fibers. I expect the former occurred. Carbon fibers were reportedly seen in the submucosa at the anterior end of the nasopharyngeal duct at all sacrifices usually with no adjacent tissue reaction. The lengths of the fibers used in our study were two orders of magnitude greater, which accounts for the lack nasal inhalability in our test. The sonic nozzle dissemination neither diminished the diameters nor the lengths of the fibers.

5. CONCLUSIONS

In this experiment, Fischer 344 rats were exposed by repeated inhalation to three concentrations of 3.5 µm diameter carbon fibers and examined at 24 hr and 14 days PE for any histopathological, physiological, bronchoalveolar lavage changes. There were no adverse changes and no evidence of fiber deposition in the nasal or pulmonary systems. This study supported the modeling data in the literature that predicted that 3.5 µm diameter carbon fibers with a large aspect ratio would not be respirable. There was no evidence that the sonic nozzle method of dissemination reduced either the diameters or lengths of the fibers.

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APPENDIX A

QUALITY ASSURANCE

This study was examined for compliance with Good Laboratory Practices as published by the U. S. Environmental Protection Agency in 40 CFR Part 792. The dates of all inspections and the dates the results of those inspections were reported to the Study Director and management were as follows:

Phase inspected	Date	Date reported
Dosing via inhalation	18 July 1989	2 August 1989
Data	14 June 1990	15 June 1990
Final Report	14 June 1990	15 June 1990

To the best of my knowledge, the methods described were the methods followed during the study. The report was determined to be an accurate reflection of the raw data obtained.

Fred K. Lee
QA Specialist, Toxicology

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APPENDIX B

SEM PHOTOGRAPHS OF RAT RESPIRATORY SYSTEM

DEPARTMENT OF THE AIR FORCE

HARRY G ARMSTRONG AEROSPACE MEDICAL RESEARCH LABORATORY (AFSC)
WRIGHT-PATTERSON AIR FORCE BASE, OHIO 45433-6573

REPLY TO ATTN OF: THT

6 November 1989

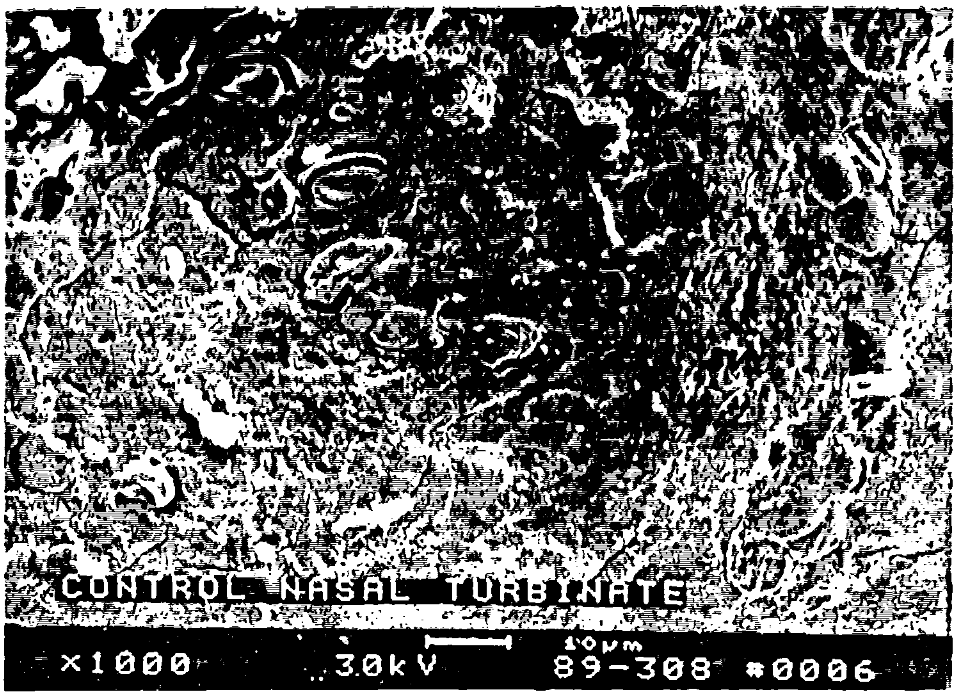
SUBJECT: SEM Photographs of Rat Respiratory System

TO: Sandra Thomson, PhD
Environmental Toxicology Branch
Toxicology Division
CRDEC
Aberdeen Proving Grounds, Maryland 21010-5423

Enclosed are representative scanning electron microscopy (SEM) photographs of the rat respiratory system after repeated exposure to carbon fibers (labeled 0-time) as well as control photographs. We can supply you with any size copies of these photographs as well as a complete set of photographs taken for the study. Except for some questionable material (less than 10 micrometers in length) found in the trachea of one rat at 0-time no fibers were found in the respiratory systems of rats exposed to 100mg/m^3 for two weeks.

David R. Mattie

David R Mattie, PhD
Chief, Ultrastructural Research Laboratory
Toxicology Branch
Toxic Hazards Division



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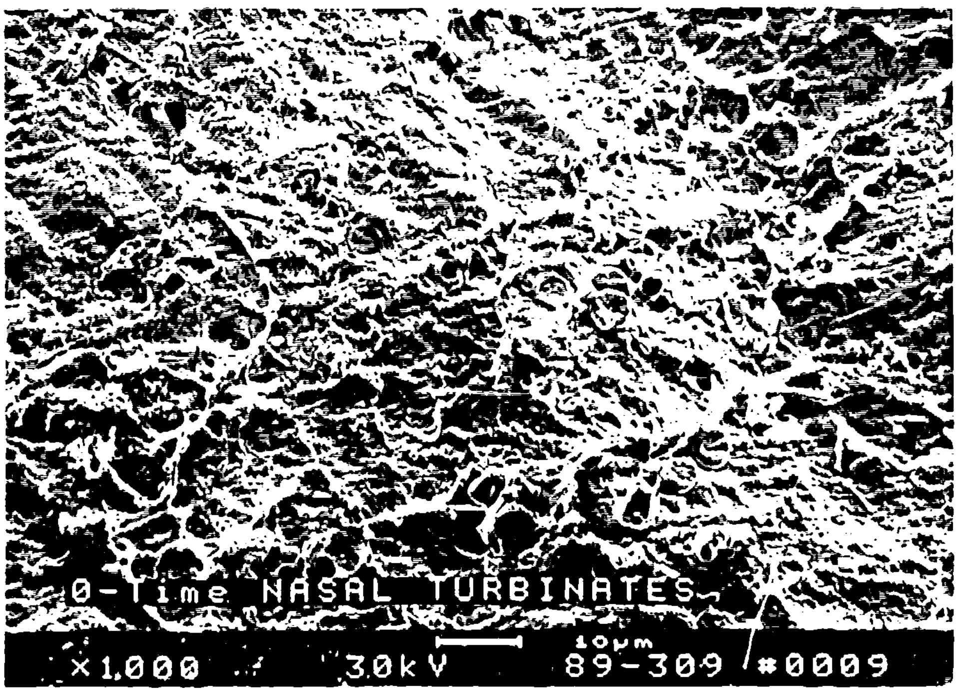
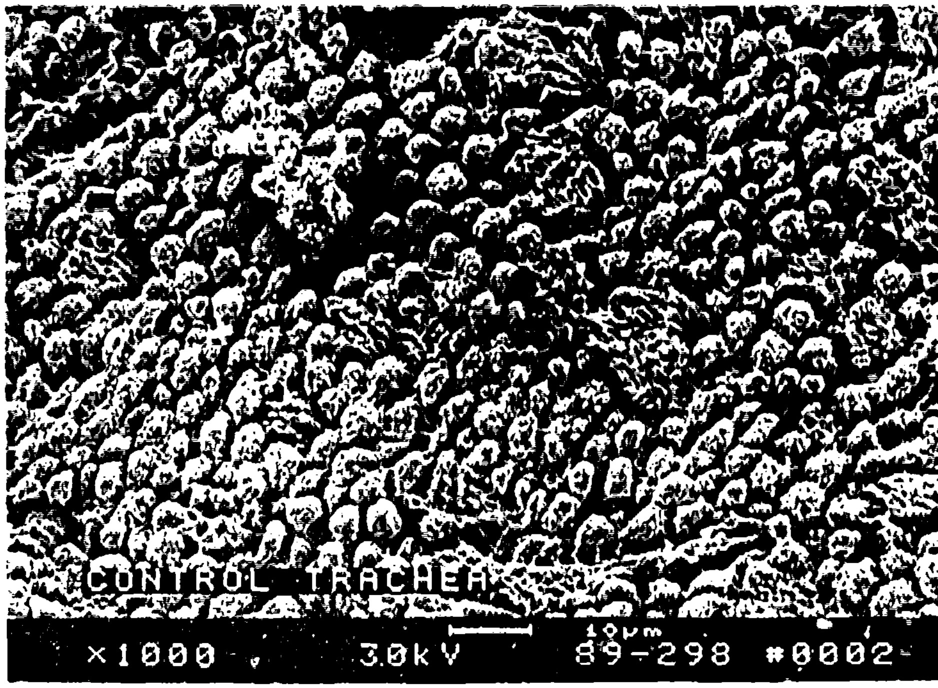
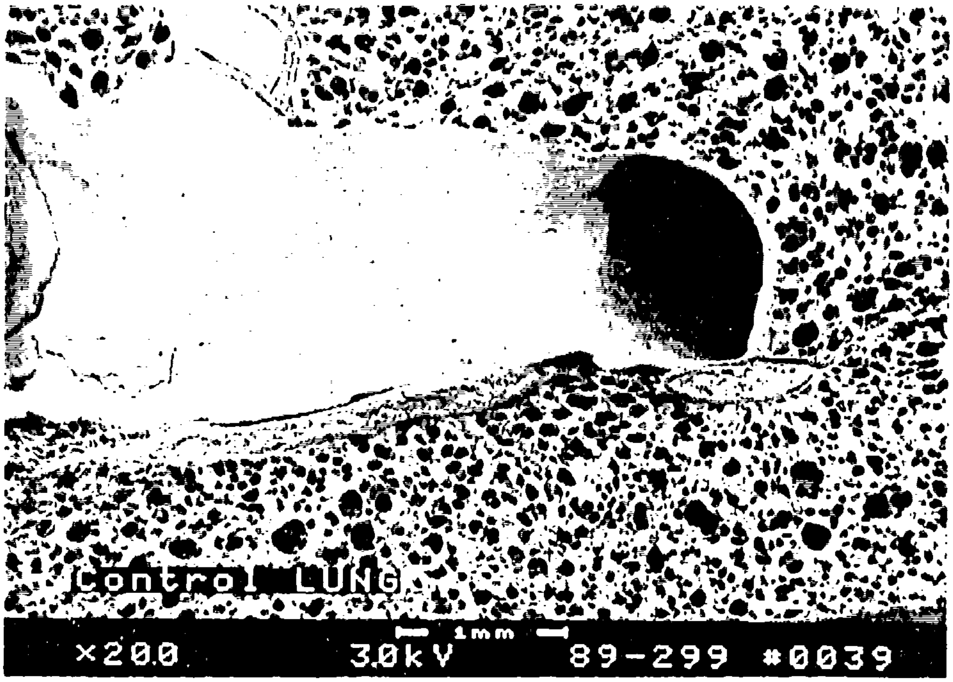


0-Time Nasal Turbinates



Control Trachea

0-Time Trachea



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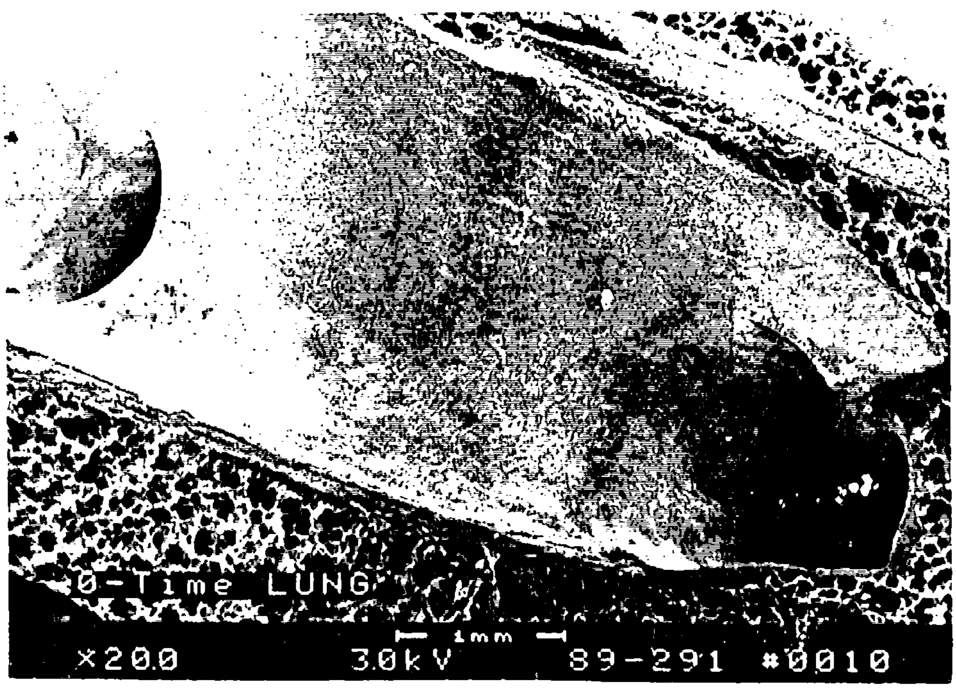

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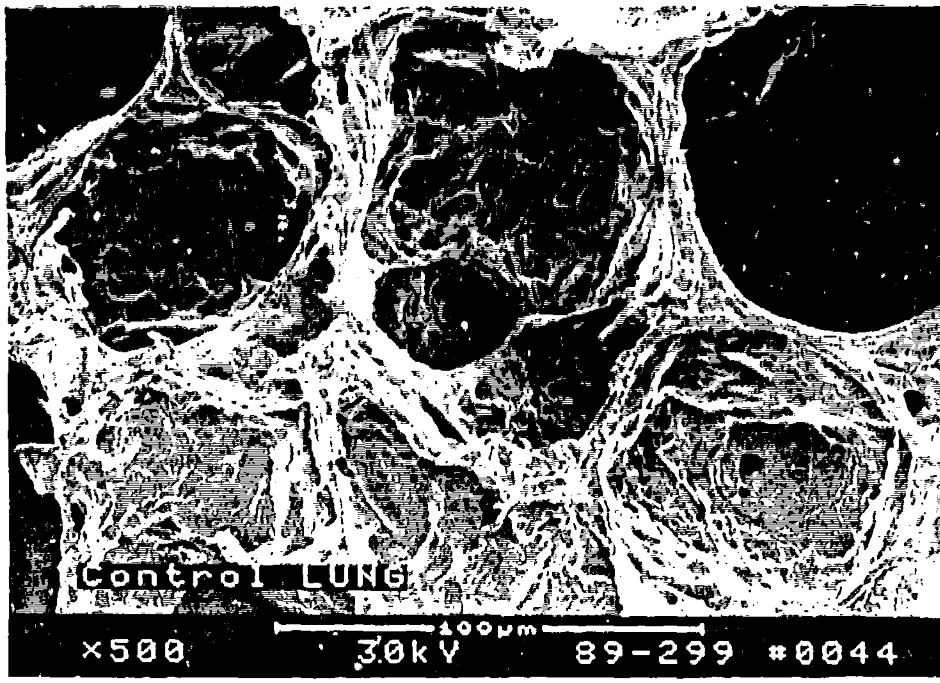
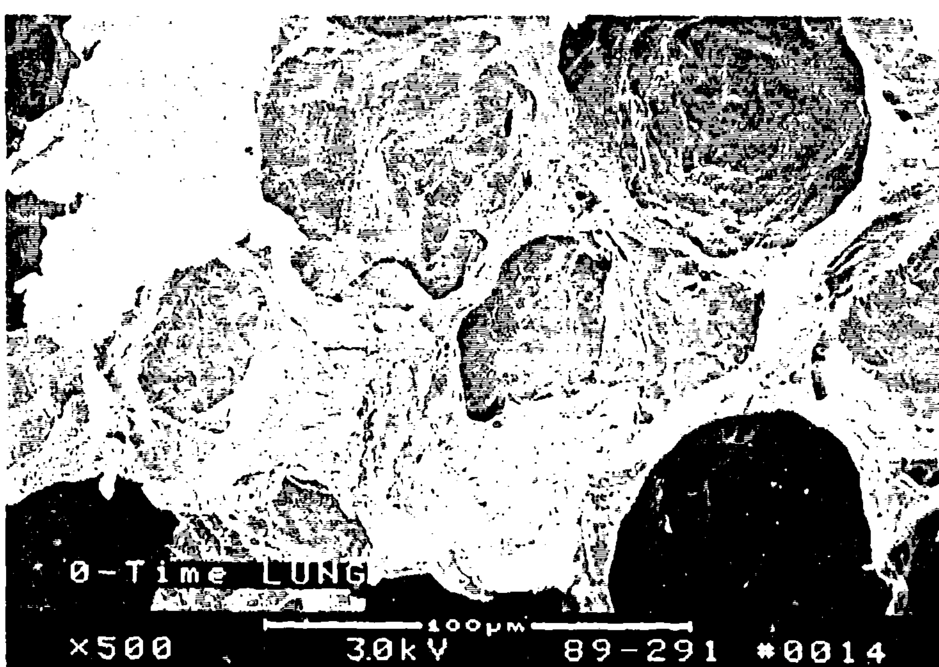


Control Lung



0-Time Lung